Available online at www.sciencedirect.com
ScienceDirect
Chemico-Biological Interactions 166 (2007) 341–351
Chemico-Biological Interactions
www.elsevier.com/locate/chembioint

Chloroprene: Overview of studies under consideration for the development of an IRIS assessment☆

Ines Pagan *

U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Mailcode B-243-01, 109 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA

Available online 12 December 2006

Abstract

Beta-chloroprene (C_4H_5Cl, chloroprene, 2-chloro-1,3-butadiene, CASRN 126-99-8) is a volatile, flammable liquid monomer utilized primarily in the manufacture of neoprene (polychloroprene) elastomer used in belts, hoses, gloves, wire coatings, and tubing. Absorption into the body occurs primarily via the respiratory system and may occur via the gastrointestinal tract or the skin. Once absorbed, chloroprene is widely distributed as evidenced by effects in several target organs including nose and lung, liver, and skin. Chloroprene metabolism is believed to include cytochrome P450 oxidation to a monoepoxide, hydrolysis by epoxide hydrolases, and glutathione conjugation. Similar to 1,3-butadiene, the epoxide is considered to be the toxic moiety, and species differences in metabolic capacity may influence the severity of effects as well as what tissues are affected.

EPA has not previously developed an assessment of chloroprene's potential for human health effects. Existing human epidemiological studies offer little data on noncancer effects, and the associations of exposure with increased cancer (liver and lung) mortality reported are inconclusive. Recent epidemiological studies (submitted for publication) could offer information that may impact chloroprene's health assessment. Multiple-site tumors have been reported in rats and mice exposed to chloroprene by inhalation; nevertheless, there are marked differences in strain sensitivities (i.e., tumors in F344 rats versus no tumors in Wistar rats). Recently developed physiologically based toxicokinetic models may allow for the resolution of species and tissue differences and sensitivities as well as exposure—dose–response relationships relevant to humans. (This presentation does not necessarily reflect EPA policy.)

Keywords: Beta-chloroprene; Monoepoxide; Human health effects

1. Introduction

Beta-chloroprene (C_4H_5Cl, 2-chloro-1,3-butadiene, CASRN 126-99-8) is a liquid monomer utilized primarily in the manufacture of polychloroprene elastomer. These materials are used to make belts, hoses, gloves, wire coatings, tubing, solvents, and adhesives [1]. Absorption and systemic distribution via the inhalation route can be inferred by toxic and carcinogenic effects in multiple target organs (nose and lung, liver, and skin) in exposed rats and mice [2]. In vitro studies suggest that chloroprene is metabolized via the cytochrome P450 enzyme system to a monoepoxide and further metabolized and cleared via hydrolysis and/or glutathione conjugation reactions. Similar to 1,3-butadiene, the epoxide is considered to be the toxic moiety. Kinetic studies in mice, rats, and hamsters suggest that species and tissue differences in metabolism may be associated with the species and sensitivity differences observed with chloroprene-induced tumors. Reviews of existing occupational and epidemiological data [3,4] have pointed out

☆ International Symposium on the Evaluation of Butadiene and Chloroprene Health, 20–22 September 2005, Charleston, SC, USA.

* Tel.: +1 919 541 2322; fax: +1 919 541 0245.
E-mail address: Pagan.Ines@epa.gov.

0009-2797/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2006.12.001

limitations in individual studies that weaken the associations between chloroprene exposure, increases in liver and lung cancer, and observed mortality. Recent epidemiological studies have not found increased tumor incidence due to chloroprene exposure [5]. The World Health Organization through its IARC Monographs Programme has classified chloroprene as a 2B carcinogen, or possibly carcinogenic to humans [3]. This classification is based on sufficient experimental evidence for the carcinogenic potential of chloroprene in animals. The IARC assessment found the published human data to be inadequate to assess the carcinogenic potential of chloroprene.

This manuscript summarizes key studies and issues under consideration for the development of an IRIS assessment for chloroprene. IRIS documents (Toxicological Review and pertinent summaries) contain information on chemical-specific hazard identification and dose–response analysis organized and integrated following Agency guidelines and established standard procedures. The first step in this process is to produce a critical analysis of the existing chemical-specific information. This manuscript summarizes key studies and issues that will influence the qualitative and quantitative assessment of health effects resulting from chloroprene exposure with greater detail describing critical studies and supporting data. The information has been organized in the following sections to resemble an IRIS Toxicological Review: (Section 2) general information on chloroprene: chemical–physical properties; (Section 3) toxicokinetic information relevant to the assessment (absorption, distribution, metabolism, and excretion); (Section 4) hazard identification: human and animal studies (cancer/noncancer effects); (Section 5) genotoxicity; (Section 6) mode of toxicity/carcinogenicity; (Section 7) summary.

The studies and information under consideration for the draft chloroprene Toxicological Review for IRIS are subject to change as the information undergoes peer and administrative review. The views expressed in this paper are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

2. Chemical–physical properties and general information on chloroprene

Beta-chloroprene (C_4H_5Cl), hereafter referred to as chloroprene, is a volatile, flammable liquid. While 90% of chloroprene is used to make the solid, polychloroprene, about 10% is converted to polychloroprene latex, a colloidal suspension of polychloroprene in water [3]. Chloroprene can be produced by dimerization of acetylene and addition of hydrogen chloride or by chlorination of 1,3-butadiene. Chloroprene is also a structural analogue of isoprene (2-methyl-1,3-butadiene) and resembles vinyl chloride as far as having a single carbon-bonded chlorine and a double-bonded carbon (alkene) backbone. Being volatile and highly reactive, chloroprene is not expected to bioaccumulate or persist in the environment. Therefore, releases to the environment would likely be from manufacturing facilities or transport of the product. Because of its high vapor pressure (174 mmHg at 20 °C), chloroprene is expected to readily evaporate from water and solid surfaces. Chloroprene vapor has an estimated ionization potential of 8.95 ± 0.05 eV, and an estimated half-life in the atmosphere of less than 20 h [6]. Reactions with OH (to produce formaldehyde), O_3, and NO_3 are the expected pathways of removal, although no experimental data exist.

Of particular relevance to any toxicological studies involving chloroprene is its propensity to oxidize and form dimers and other oxygenated species unless stabilizers are added. Uninhibited chloroprene must be stored under nitrogen at below 0 °C. When bulk chloroprene with 5% *n*-octane added as an internal standard was stored at 55 °C for up to 6 h, dimer content increased 62% and chloroprene monomer decreased 22% [7]. Because these reaction products, if formed, may themselves account for the observed toxicity, results of toxicological studies that do not report storage or generation conditions may be suspect regarding the chloroprene monomer. A discussion of the polymerization process has been reported by Lynch [1], Nystrom [8], Stewart [9], and in the Kirk-Othmer Encyclopedia of Chemical Technology [10]. IARC [3] has reported additional information on production and use.

In addition to volatilization, the potential fate of chloroprene that is released to soil is to leach into groundwater. Breakdown via hydrolysis is not likely, as it is only partially soluble in water. Chloroprene that is released into water may only moderately adsorb to suspended sediments or particles, and there will be little bioaccumulation in aquatic organisms. The occupational exposure potential to chloroprene is confined to facilities in the United States, Europe, and Asia where chloroprene is produced and converted to polychloroprene [1].

3. Toxicokinetic information relevant to the assessment (absorption, distribution, metabolism, and excretion)

The information on the absorption, distribution, and in vivo metabolism and excretion of chloroprene and/or

its metabolites is nonexistent for humans and limited for animals. Several authors have proposed a metabolic pathway for chloroprene based on inferences from the toxicological database or on studies using in vitro microsomal preparations from human and animal tissues. Haley [11] proposed a biotransformation pathway for chloroprene based upon oxidation by mixed function oxidases (cytochromes P450), drawing from possible similarities to vinyl chloride metabolism, to an epoxide and possible further metabolism to aldehydes and mercapturic acid derivatives along this pathway. Bartsch et al. [12] provided evidence for the formation of alkylating intermediates of chloroprene metabolism by P450 enzymes in rats and humans. In agreement with Haley and Bartsch, studies by Cottrell et al. [13], Himmelstein et al. [14], and Munter et al. [15] have proposed and/or verified the oxidation of chloroprene to two epoxides, the more stable monoepoxide [(1-chloroethenyl)oxirane] and the less stable 2-chloro-2-ethenyloxirane in the metabolism pathway for chloroprene in rodents and humans. The chloroprene metabolic profile appears to be qualitatively similar across species; however, interspecies quantitative differences that could influence the sensitivities of chloroprene-induced effect have been investigated [15].

Himmelstein et al. [16,17] conducted further kinetic studies and developed a lung-specific dose–response model to simulate an external concentration of chloroprene for humans that would be equivalent to doses that induce lung tumors in rodents. Further development of this model to explore differences in toxication/detoxification of chloroprene across species would be useful in human risk assessment [18].

4. Hazard identification: human and animal studies (cancer/noncancer effects)

4.1. Human database

Several occupational studies and case reports of chloroprene exposure have been reported for the time period of 1950–2000. This database was reviewed by IARC [3] and summarized in Rice and Boffetta [4] and was consistently found to be inadequate to assess the carcinogenic potential of chloroprene in humans. In addition to limitations in the experimental design (e.g., no exposure information, deficient follow-up), coexposure with contaminants of the elastomer-generation process, including the carcinogen vinyl chloride, is possible. This section contains a brief review of the aforementioned database focusing on the reported chloroprene-induced effects and the major weaknesses of the studies. See Table 1 for a summary of studies, effects observed, and some of the reported limitations of the available reviews.

Sanotskii [19] summarized a Russian occupational study of reproductive effects among male chloroprene workers. The exposed cohort was reported to exhibit “disturbed” sperm function and morphology, as well as increased incidences of spontaneous abortion among the workers’ wives. This study has been questioned because of its inadequate reporting of experimental details and because no subsequent studies have replicated these observations [20,21]. It is also unclear whether these workers were involved in chloroprene production or in the use of polychloroprene. Furthermore, there was poor information on chloroprene storage con-

Table 1
Human database summary: reported effects and study limitations

Reference	Reported Effects	Limitations
Roeleveld et al. [22]	Teratogenic effects	Inconclusive
Sanotskii [19], occupational study in Russia, 143 males, 118 unexposed	Reproductive effects. Disturbed sperm function and morphology and spontaneous abortions	Information was not reproduced in other studies, deficient information on coexposure with other compounds
Shouqi et al. [30], cohort of 1258 employees in the People’s Republic of China	Liver cancers and cancer deaths	Selection criteria for cohorts unclear; bias from other experimental design weaknesses
Pell [33], historical prospective study of workers in the neoprene industry in two U.S. plants	Urinary cancers in cohort 1. Digestive tract and urinary cancers in cohort 2	No statistical trend observed after study review
Bulbulyan et al. [32], cohort study among 5185 shoe manufacturing workers (4569 were women), Moscow, Russia	Excess mortality from liver and kidney cancers	Possible coexposure with benzene, ethyl acetate, leather dust, or formaldehyde. No confirmed pathology. Some trends of increased cancers in the control group
Romazini et al. [35], retrospective cohort and a nested case–control study of French workers (599 males and 61 females) for 2 years	Some mortalities were reported	Low number of mortalities (32 out of 642 workers; 18 were lost to follow-up), incomplete account of confounding factors
Marsh et al. [5], historical cohort study	Negative for lung and liver tumors	

ditions, analytical techniques, polymer production, and chemical characterization, and this report was considered unreliable. Roeleveld et al. [22] reviewed studies that examined neurodevelopmental toxicity in children of parents occupationally exposed to various chemicals, including chloroprene. However, the association between chloroprene exposure and teratogenic effects was deemed inconclusive. Alopecia (hair loss) from the scalp has been reported among men occupationally exposed to chloroprene during the manufacture of polychloroprene [23,24]. This effect was reported to be temporary and reversible upon cessation of exposure [25]. Alopecia was also a concentration-related effect observed in Wistar rats exposed to chloroprene [26]; this study is further described in the animal database section.

A report by Dong et al. [27] cited a study conducted in China that involved positive micronucleus tests of peripheral blood erythrocytes in chloroprene workers. Gooch and Hawn [28] reported changes in blood chemistry parameters (serum glucose, cholesterol, and lactate dehydrogenase) on workers at a chloroprene polymerization plant; however, the exposure levels on the exposed and control groups from this study were unclear. Ward et al. [29] examined potential hepatoxicity among chloroprene/polychloroprene production workers at a chemical plant in Texas. Indices of hepatic function were assessed including liver enzyme activities (serum glutamate-oxaloacetate transaminase, serum glutamate-pyruvate transaminase, γ-glutamyl transpeptidase, alkaline phosphatase, cholinesterase), bilirubin levels (total and direct) and determination of prothrombin time. The authors reported that 4 individuals out of 81 in the sample exhibited clinically significant abnormalities; however, the increases in liver enzyme activities were reported to be related to alcohol consumption. The authors noted a trend toward increased values among workers in the "high exposure areas" and concluded that the results suggest that exposure to chloroprene may contribute to liver function abnormalities and that individuals who consume alcohol may be particularly at risk. This study was also limited by a lack of adequate exposure data and the possible coexposure of workers to chemicals other than chloroprene.

Shouqi et al. [30] described a preliminary trial that included both a case–control and cohort analyses with data on cancer deaths occurring between 1969 and 1983 among chloroprene/polychloroprene (neoprene) production workers in China, most of whom were exposed to chloroprene beginning in 1952. Wage roll workers were categorized according to likely exposure to chloroprene as determined by their occupation and the opinions of workers and administrators as to levels of exposure. An increased risk of cancer death was reported to be associated with chloroprene exposure, with the average age at death less than that of unexposed workers. Increased incidences in liver and lung cancers and of malignant lymphomas were reported. Causes of death were abstracted from medical records; however, it appears that there were no histologic confirmation of the diagnoses.

While this study raised concern for a link between exposure to chloroprene monomer and multisite cancer mortality, other causes or contributing factors (such as alcohol consumption and smoking) cannot be ruled out. Another confounder is possible coexposure to chloroprene oligomers in this study. Thus, the reported associations were regarded as inconclusive.

In a retrospective cohort study of 2314 workers (1897 men, 417 women) employed in an Armenian chloroprene monomer production plant between 1940 and 1988, Bulbulyan et al. [31] found a duration-of-exposure-related increase in liver cancer compared to the overall Armenian population. Four of six cases of liver cancer occurred in workers with 20+ years of employment. The total cohort was followed for cancer incidences between 1979 and 1990 and for mortality from 1979 to 1988. Causes of death were abstracted from death certificates and classified according to the ninth revision of the International Classification of Diseases. Before 1980, measured air levels ranged from about 0.2 ppm to over 200 ppm. After 1980, a maximum air level was reported at ∼6 ppm. Study limitations that preclude a positive association between exposure to chloroprene and liver cancer include (1) lack of follow-up prior to 1979, which could seriously bias the incidence ratios; (2) lack of accounting for alcohol use; (3) lack of histologic confirmation; (4) possible coexposure to other chemicals. Another study by Bulbulyan et al. [32] examined cancer mortality in Moscow shoe workers exposed to chloroprene from glue and from polychloroprene latex (a colloidal suspension of polychloroprene in water). The extent to which the subjects were exposed to chloroprene monomer and the analytical methods used were not discussed. The study comprised a total of 5185 workers (4569 of which were women) employed for at least 2 years during 1960–1976 and followed up during 1979–1993. Workers were assigned to three exposure groups based on industrial hygiene data from the 1970s: no exposure, medium exposure (0.1–0.2 ppm), and high exposure (6 ppm). Causes of death were abstracted from death certificates and classified according to the ninth revision of the International Classification of Diseases. There were no histologic confirmation of causes of death. A total of 131 workers were lost to follow-up. The

authors found the mortality due to all cancers was higher than expected. Significantly increased mortalities were also seen for liver cancer and leukemia. In addition, lung cancer was increased in men but not in women. There were elevated relative risks observed among the medium exposure group for stomach, liver, and kidney cancers and among the high-exposure group for stomach, liver, kidney, pancreas, and colon cancers and leukemia. When analyzed according to employment duration (1–9 years, 10–19 years, 20+ years), a significant linear trend was seen in mortality rates from liver cancer and leukemia (only in the high-exposure group) with increased duration of employment. Limitations of this study include (1) possible confounding from coexposure of workers to benzene and other chemicals during part of their employment, (2) lack of reliable data on chloroprene and polychloroprene exposure levels throughout the entire study period, and (3) lack of control for smoking and alcohol use. Similar to other reports, possible associations between exposure to chloroprene monomer and cancer are inconclusive.

Pell [33] examined cohorts from two polychloroprene manufacturing plants to evaluate lung cancer mortality due to chloroprene exposure. Increased deaths from lung cancer and cancer of the lymphatic and hematopoietic systems were observed in one cohort. In the second cohort, cancers of the bladder and kidney were observed. The bladder cancers observed in this cohort were attributed to β-naphthylamine exposure. A reanalysis of this study by Leet and Selevan [34] concluded that there were no statistically significant trends in numbers of deaths from malignant neoplasms either for latency or duration of exposure. The investigators stated that the statistical power of the study was limited because of cohort selection factors and stratification.

Romazini et al. [35] investigated the occurrence of cancer deaths in a retrospective cohort and a nested case–control study of French workers (599 men, 61 women) for 2 years (between 1966 and 1989) in a polychloroprene plant. Some mortalities were reported; however, the low number of mortalities (32 out of 642 workers; 18 were lost to follow-up), unsubstantiated exposure groupings, and incomplete accounting of confounding factors make this study inconclusive.

More recent epidemiological studies have not found an association between chloroprene exposure and increased incidences of cancers of liver or lung [5].

4.2. Animal database

Chloroprene's toxic and carcinogenic potential have been assessed in two chronic inhalation bioassays, one in rats and mice conducted by the National Toxicology Program (NTP) [2] and another in rats and hamsters conducted by Trochimowicz et al. [26].

Sixteen-day and 13-week range-finding studies were conducted prior to the chronic 2-year bioassay [2,36,37]. Brief summaries of chloroprene-induced effects focusing on target organs and the exposure levels at which these were observed are presented below. All exposure regimes consisted of 6-h whole-body exposures each day, 5 days per week, with group sizes of 10 animals per sex per group in the 16-day and 13-week studies, and 50 animals per sex per group in the 2-year studies. The actual chloroprene exposure concentrations were within 99% of target concentrations. There was no degradation of bulk chemical (stored under nitrogen at −20 °C), and the total impurities in the distribution line during exposure was less than 0.1%.

In the 16-day study, rats were exposed to chloroprene at 0, 32, 80, 200, or 500 ppm (0, 115, 288, 720, or 1800 mg/m^3). Target organs affected include the nervous system, nose and respiratory system, hematopoietic system, liver, and kidney. Mean body weights were decreased in males (500 ppm) and females (200 ppm). All rats exposed to 500 ppm, and some in the 200-ppm group, were reported to be hypoactive, unsteady, and presenting rapid, shallow breathing on the first day of the study. These effects worsened and hemorrhage from the nose was observed. Three males died at 500 ppm. Clinical chemistry revealed responsive anemia, thrombocytopenia, and increases in serum enzymes (alanine aminotransferase, glutamate dehydrogenase, and sorbitol dehydrogenase) in males and females in the range of 200–500 ppm. Females had increased kidney and liver weights starting at 80 ppm. Centrilobular-to-random hepatocellular necrosis was observed in males and females at 200 or 500 ppm. At all exposure concentrations both males and females had mild olfactory epithelial degeneration. Males had increased squamous metaplasia of the respiratory epithelium at 500 ppm. In the subsequent 13-week range-finding study, rats were exposed to 0, 5, 12, 32, 80, or 200 ppm (0, 18, 43, 115, 288, or 720 mg/m^3). Consistent with the 16-day study, target organs affected included the nose and respiratory system, hematopoietic system, nervous system, liver, and kidney at 80 or 200 ppm. There were no changes in body weight in any of the exposed animals. Males had clear or red discharge around the nose and eyes at 200 ppm. In neurobehavioral tests, total activity in males was decreased (starting at 32 ppm). Incidences of epithelial olfactory degeneration and respiratory metaplasia were increased in males (80 or 200 ppm) and females (32, 80, or 200 ppm). Chronic liver inflammation in males

and females, and hepatocellular necrosis in females, was observed at 200 ppm.

In the 16-day study, mice were exposed to chloroprene at 0, 12, 32, 80, and 200 ppm (0, 43, 116, 290, 724 mg/m^3). Target organs affected included the nervous system, nose, heart, thymus, liver, and forestomach. All males and females exposed to 200 ppm presented narcosis, were hypoactive, and had reduced body tone in the first day of exposure and died on days 2–3 of the study. Pathological findings in the high-dose group included multifocal random hepatocellular necrosis, myocardial hypertrophy, hemorrhage and mucosal erosion, and thymic necrosis (200 ppm, males and females). At lower doses, mean body weights were decreased in males (32 or 80 ppm) along with reduced thymus (80-ppm males and females) and liver (80-ppm females) weights and squamous epithelial hyperplasia of the forestomach (80-ppm males and females). In the subsequent 13-week range-finding study, mice were exposed to chloroprene at 0, 5, 32, or 80 (0, 18,115, or 288 mg/m^3). All animals survived to the end of the study. Effects observed included decreased mean body weights in males (80 ppm), decreased hematocrit concentration (32 or 80 ppm) and erythrocyte counts (80 ppm) in females, increased platelet counts (32 and 80 ppm) in females, and increased incidences of squamous epithelial hyperplasia of the forestomach in males and females (80 ppm).

In the 2-year study, F344 rats and B6C3F1 mice were exposed to 0, 12.8, 32, and 80 ppm (0, 46, 116, and 290 mg/m^3) chloroprene by inhalation. Animals that died during the study or were killed at the end of the exposure period received a complete necropsy and histopathological examination.

In rats, chloroprene-induced effects were observed in the oral cavity, thyroid gland, lung, kidney, and mammary gland. Survival in the 32- and 80-ppm groups of males was significantly lower than control, while survival of female rats was not affected. Body weight gain was not significantly reduced over the span of the study. Concentration-dependent increases in the incidence of squamous cell carcinoma and papilloma of the oral cavity were observed in both males and females (80 ppm). Follicular cell adenoma or carcinoma of the thyroid for females showed less of a concentration dependence than for males; statistical significance was achieved in males for the combined effects at both 32 and 80 ppm. The incidence of alveolar/bronchiolar carcinoma in males reached statistical significance at 80 ppm with little indication of a concentration-related trend, while the incidence of hyperplasia of the alveolar epithelium (in both sexes) was statistically significant at all exposure concentrations compared to controls. An increase (not statistically significant) in the incidence of alveolar/bronchiolar adenomas was seen in females exposed to 80 ppm that exceeded the incidence in historical controls, but no adenomas were seen in the two lower-concentration groups. There were no carcinomas. In females, the incidences of multiple fibroadenomas in the mammary gland of all exposed groups were greater than controls.

The incidence of renal adenomas/carcinomas was significantly greater than controls in males from all exposure groups. In the urinary bladder, there was a slight increase in transitional epithelium carcinoma in females at 80 ppm and in males at 32 ppm. In addition, 1/50 males at 80 ppm had a transitional cell papilloma. All these incidences exceeded the historical control ranges; no such neoplasms have been observed in historical controls in rats. The findings in the bladder were considered by the NTP to be of uncertain significance although noteworthy, because no such neoplasms have been seen in either male or female control F344/N rats. The NTP concluded that, overall, there was clear evidence of carcinogenicity.

Prominent among nonneoplastic lesions were atrophy, basal cell hyperplasia, metaplasia, and necrosis of the olfactory epithelium in 32- and 80-ppm males and females. Atrophy and necrosis were elevated in males in all exposure groups and in females only in the two highest concentration groups.

In mice, chloroprene-induced neoplastic effects were observed in the lung, circulatory system (hemangiomas and hemangiosarcomas), liver, forestomach, Harderian gland, kidney, mesentery, Zymbal's gland, and mammary gland. Survival of females was significantly lower than controls in all exposure groups and in the two highest-exposure groups of males. Many early deaths and moribund sacrifices were stated to be associated with treatment-related neoplasms. Although there was an increased incidence of adenomas, carcinomas, and hemangiosarcomas of the liver in male mice, these lesions were judged to have been influenced by *Heliobacter hepaticus* infection, which may have resulted in hepatitis. Increased incidence of hepatocellular carcinoma was seen in all exposed females but was not considered to be a result of Heliobacter infection. An increased incidence of Zymbal's gland carcinoma, which metastasized to the lung, was seen in the 80-ppm females. Nonneoplastic effects reported in mice included (1) increased Incidence of bronchiolar hyperplasia (at all concentrations) and histiocytic cell infiltration in the lung (mainly in the high-concentration group), (2) epithelial hyperplasia in the forestomach (high-concentration group only), (3) renal tubule hyperplasia (males only; no

concentration-related response), and (4) atrophy of the olfactory epithelium (high-concentration group only).

Trochimowicz et al. [26] conducted a chronic study evaluating the carcinogenic potential of chloroprene. In the preliminary 4-week range-finding assay, male and female Wistar rats were exposed to mean concentrations of 0, 39, 161, or 625 ppm (0, 140, 580, 2250 mg/m^3) freshly distilled chloroprene for 5 days per week, 6 h per day. While no mortality was seen in the control and 39-ppm groups, three males died by week 4 in the 161-ppm group, and five males and three females died in the 625-ppm group. Gross pathology of animals that died included dark, swollen livers. Survivors of the high-exposure group had grayish lungs with hemorrhagic areas. Mean body weights were significantly lower than controls in all exposure groups beginning at the first week of exposure; retarded growth reflected an exposure-related trend. Significant concentration-related decreases in liver and spleen-to-body weight ratios were seen; brain-to-body ratios increased across exposure groups. Microscopic examination revealed centrilobular liver degeneration and necrosis, slightly enlarged tubular epithelial cells in kidneys, and hemorrhage and edema in the lungs of animals in the high-exposure group. There were no adverse liver, kidney, or lung effects in the 39-ppm group. There were no adverse hematological findings in any exposure group.

In the lifetime exposure assay, Wistar rats and Syrian golden hamsters (100 animals per sex per group) were exposed to chloroprene at 0, 10, and 50 ppm (0, 36, or 181 mg/m^3), for 6 h per day, 5 days a week for 24 months or for 18 months, respectively. Stock solutions of freshly distilled chloroprene were stored under nitrogen at −20 °C and vapors were generated from vessels kept at 0 °C. Purity was 99.6% β-chloroprene. Histological examination of liver, spleen, pituitary, thyroid, adrenals, and tumors were conducted on animals in the lower-exposure groups. Clinical chemistry was not part of the protocol, and hematological and immunological assessments were not made. At week 72, a chamber failure caused the accidental deaths of 87 male and 73 female rats in the 10-ppm group. Mortality rates in the 50-ppm group were similar to controls. Rats in the 50-ppm group exhibited (1) a concentration-related increase in the severity of, and an increased incidence of, alopecia (greater in females than in males); (2) increased relative liver weight (females only); (3) lower relative spleen and thyroid weights (females only); (4) decreased lung weight (both sexes) with 10% growth retardation (i.e., body weight gain) and increased incidence of clear hepatocellular foci (males) and a combination of basophilic, clear, and mixed-cell type foci in females. The livers of the 10-ppm group rats that died accidentally were slightly-to-moderately autolytic, precluding histological findings. There were no statistically significant compound-related effects on the kidney, spleen, and thyroid of either sex.

Statistically significant neoplastic findings in this study included an increase in mammary fibroadenomas in female rats and an increase in squamous-cell carcinomas of the skin in male rats, both at 50 ppm. The incidence of thyroid follicular adenomas in females in the 50-ppm group was 3/100, while the incidence of papillary carcinoma was 2/100; no neoplasms were found in the thyroid for female controls. Papillary carcinoma was not observed in male rats. The incidence of Zymbal's gland adenoma was 1/100 in 50-ppm group females. The incidence of nasal squamous-cell carcinoma in males of the 50-ppm group was 3/100; one such carcinoma was found in a control-group female. The incidence in males was reported to be within the historical range (0–3.4%) for Wistar rats; therefore, the investigators concluded that the occurrence of this neoplasm was not treatment-related. The incidence of transitional-cell carcinoma of the urinary bladder was 1/100 males in the 50-ppm group. Alopecia was observed in rats exposed to 50 ppm and was categorized by the authors as likely to be a compound-related event.

In hamsters, there were no remarkable differences in gross or microscopic pathology nor increases in neoplasms reported to be statistically significant.

Trochimowicz et al. [26] concluded that chloroprene is not carcinogenic in either rats or hamsters under these exposure conditions. This conclusion for the rat differs considerably from the findings in the NTP study in which multisite tumors were observed in the high-concentration group. The investigators proposed that this may relate to the difference in the chloroprene exposure concentration or to species and/or strain differences.

5. Genotoxicity

Chloroprene has been primarily negative in in vitro and in vivo genotoxicity assays (Table 2). Negative results have been reported for the following assays: bacterial mutations in Salmonella strains, sister chromatid exchange, chromosomal aberrations, and micronuclei frequency in either polychromatic or normochromatic erthyrocytes [2,38]. Other negative results were observed with micronucleated cells in peripheral blood erythrocytes of mice exposed to chloroprene for 13 weeks [6]. Sanotskii [19] reported an increase in chromosomal aberrations in bone marrow cells of mice exposed for 2 months to chloroprene concentrations of 1 ppm or

Table 2
Genetic toxicology of chloroprene

Type of test	Result	Reference
Chloroprene (parent compound tested): *Salmonella typhimurium*, TA 98, 100, 1535, 1537	Negative	NTP [2]
Sex-linked recessive lethal mutation: *Drosophila megaloblaster*	Negative	NTP [2]
Sister chromatid exchange: mouse bone marrow cells (in vivo)	Negative	NTP [2]
Chromosomal aberrations: mouse bone marrow cells (in vivo)	Negative	NTP [2]
Micronucleated erythrocytes: mouse peripheral blood (in vivo)	Negative	NTP [2]
Chromosomal aberration: mouse bone marrow cells	Positive	Sanotskii [19][a]
Micronuclei assay: mouse bone marrow cells (in vivo)	Negative	Shelby and Wit
Recessive lethal mutation assay		
D. megaloblaster	Positive	Vogel [40][a]
S. typhimurium	Positive/negative (depending on bacterial strain and purity of test compound)	Westphal et al. [39][a]
S. typhimurium, TA 100	Positive/negative when purified solution tested	Bartsch et al. [12]
Chloroprene metabolite tested: 1-chloroethenyl(oxirane)	Positive	Himmelstein et al. [41]
Micronuclei: Chinese hamster V9 cell culture	Negative	Himmelstein et al. [41]

[a] Equivocal positive results related to contaminants present in the exposure system in addition to the chloroprene monomer.

less; however, protocol details and information about the purity and storage of chloroprene were not provided. As shown in the experiments with *Salmonella typhimurium* by Westphal et al. [39], chloroprene in DMSO as a solvent showed markedly more toxicity and mutagenicity than when in the solvents ethanol or xylene. Other positive tests including a lethal mutation assay in *Drosophila melanogaster glbl* [40] and an *S. typhimurium* assay [39] are considered equivocal due to the presence of decomposition products such as chloroprene cyclic dimers in the test medium, which may have been responsible for the observed toxicity. Indeed, Westphal et al. [39] reported that freshly distilled chloroprene (from a 50%-in-xylene solution) was negative in the Ames assay with and without S9. This study showed that the mutagenicity of aged chloroprene increased linearly with age of the distillate. Further analysis of the aged chloroprene by gas chromatography revealed the presence of decomposition products, including cyclic dimers.

Himmelstein et al. [41] demonstrated that the proposed putative chloroprene metabolite (1-chloroethenyl) oxirane was mutagenic in the *S. typhimurium* bacterial assay system; however, it was found not to be clastogenic in cultured Chinese hamster cells.

6. Mode of toxicity/carcinogenicity

One of the key features of EPA's new Cancer Guidelines [42] is the emphasis on the use of the mode(s) of action (MOA) framework to characterize the steps along the continuum to toxicity/carcinogenesis. The MOA is defined as "key events and processes, starting with the interaction of an agent with a cell, through functional and anatomical changes, resulting in cancer or other health endpoints," with those key events being the necessary element(s) to that MOA. In order to reach conclusions on a hypothetical MOA, one must have sufficient support from the chemical-specific database in animals (in the absence of adequate evidence in humans) and ascertain its relevance to humans.

The proposed metabolic pathway for chloroprene includes oxidation by P450 enzymes (likely to be CYP2E1) to the epoxide (1-chloroethenyl)oxirane, and possibly 2-chloro-2-ethenyloxirane, hydrolysis by epoxide hydrolases and conjugation with GSH. Aldehydes and ketones are additional proposed intermediates.

Results from comparative metabolic in vitro studies using liver and lung microsomes from a variety of laboratory animals (including rat, mouse, and hamster) and humans suggest that the metabolic pathway for chloroprene is qualitatively similar across these species. However, there are quantitative species-specific differences in metabolism and in putative metabolite stereochemistry that, if operative in vivo, could lead to differences in the sensitivities of humans and rodents. Recent dose–response models [43] may assist in the elucidation of the biological significance of these findings.

Epoxide intermediates, such as those proposed for the oxidative metabolism of chloroprene, have been shown to interact with DNA and/or proteins, in vitro, and thus may play a role in the toxicity/carcinogenicity of this compound. However, chloroprene (the parent

compound) has been primarily negative in in vitro and in vivo genotoxicity assays. Contradictory positive mutagenicity results may be due to the presence of either decomposition products such as chloroprene cyclic dimers or reactive compounds (vehicle DMSO) in the test medium.

The proposed putative chloroprene metabolite (1-chloroethenyl)oxirane has been found to be mutagenic in vitro, however, it has been negative in vivo.

The in vitro reactivity of (1-chloroethenyl)oxirane with hemoglobin (adduct formation) and enantiomer detoxification (i.e., disappearance of *R*-enantiomer versus *S*-enantiomer from the test system) in vitro have been investigated by Hurst and Ali [44]. Mouse (C57BL/6) erythrocytes (RBCs) were incubated with the *R*- and *S*-enantiomers of (1-chloroethenyl)oxirane in vitro. The authors reported a greater persistence of the *R*- over the *S*-enantiomer upon incubation with RBCs in the in vitro system tested. The authors also reported a relative greater amount of globin adducts formed with the *R*- than with the *S*-enantiomer. Pretreatment with a glutathione (GSH)-depleting compound (diethyl maleate) increased the persistence of the *S*-enantiomer (substantially) and the *R*-enantiomer (modestly). Given these data, the investigators proposed a mechanism of enantiomer selective detoxification [persistence of *R*- versus disappearance of *S*-(1-chloroethenyl)oxirane enantiomers] by mouse RBCs involving the enzymatic activity of glutathione *S*-transferase. Further in vivo studies of identification and measurement of chloroprene epoxide adducts and detoxification are needed to complement and provide support for such in vitro findings.

A qualitative schema incorporating kinetic and dynamic processes that would inform the chloroprene MOA is illustrated in Fig. 1. Further investigations are needed to characterize the key events in the chloroprene MOA.

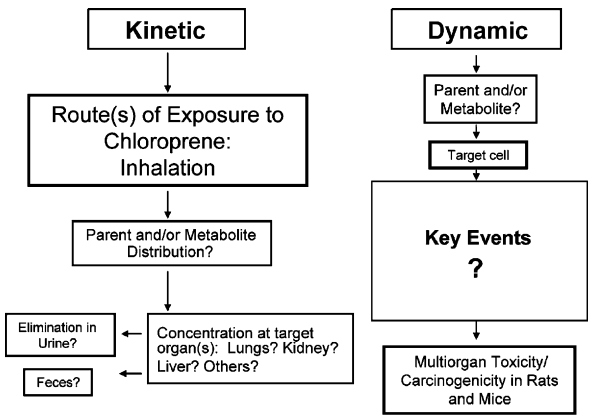

Fig. 1. Chloroprene mode of toxicity/carcinogenicity schema of kinetic and dynamic events.

7. Summary

Chloroprene has been identified as a potential human carcinogen because of its structural similarity to known human carcinogens butadiene and vinyl chloride. Early occupational epidemiological studies and case reports that were reported in 1950–2000 have been reviewed by IARC [3] and summarized by Rice and Boffetta [4] and have been consistently found inadequate to assess the carcinogenic potential of chloroprene in humans. Some of these inadequacies include lack of exposure information, deficient methodology, deficient follow-up on cohorts, lack of histopathological confirmations of effects, and cancers attributed to coexposure with other carcinogens, i.e., vinyl chloride. Nevertheless, tumors resembling those observed in the animal chronic studies were reported in those early human studies (i.e., liver, lung, and urinary tract cancers). In addition, chloroprene-induced effects such as dermatitis and hair loss have been observed in both human and animal studies.

More recent epidemiological studies have not found positive associations between chloroprene exposure and cancers of the liver and lung [5].

More experimental information is needed to clarify the in vivo absorption, distribution, metabolism, and elimination of chloroprene. Absorption and systemic distribution via the inhalation route can be inferred by toxic and carcinogenic effects in multiple target organs in exposed rats and mice [2,36,37]. Inhalation uptake and metabolism has been confirmed in rats and mice by in vivo gas uptake exposure and treatment with the cytochrome P450 inhibitor, 4-methylpyrazole [17]. In vitro studies suggest that chloroprene is metabolized via the cytochrome P450 enzyme system to a monoepoxide [(1-chloroethenyl)oxirane] and further metabolized and cleared via hydrolysis and/or glutathione conjugation reactions. Similar to 1,3-butadiene, the epoxide is considered to be the toxic moiety. The metabolic profile for chloroprene appears to be qualitatively similar across species. However, in vitro kinetic studies using tissues from rodents and humans suggest that quantitative species and tissue differences in metabolism could be associated with the species, strain (in rats), and gender differences observed in chloroprene-induced tumors. Recent kinetic studies and models may help evaluate the nature of these differences across species and gender.

The genotoxicity data for chloroprene is primarily negative. The proposed putative metabolite, (1-chloroethenyl)oxirane has been shown to be mutagenic

[41], and in vitro studies have shown hemoglobin adduct formation with its *R*- and *S*-enantiomers [44]. However, no experimental in vivo evidence exists on DNA and/or protein reactivity in blood or target tissues. Thus, further data are needed for a better characterization of the mode of action leading to the observed toxicity/carcinogenicity of chloroprene.

As previously stated, the information discussed in this manuscript is under consideration for the draft chloroprene Toxicological Review for IRIS and is subject to change as it undergoes peer and administrative reviews. The views expressed in this paper are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

Acknowledgements

Sincere thanks are due to Gary Foureman, Mark Greenberg, and Robert Dewoskin for their careful review of this manuscript.

References

[1] M. Lynch, Manufacture and use of chloroprene monomer, Chem. Biol. Interact. 135/136 (2001) 155–167.
[2] NTP, Toxicology and Carcinogenesis Studies of Chloroprene (CAS No. 126-99-8), in F344/N Rats and B6C3F1 Mice (Inhalation Studies), National Toxicology Program, NTP TR 467, 1998.
[3] International Agency for Research on Cancer (IARC), IARC Monograph, vol. 71: Re-evaluation of some Organic Chemicals, Hydrazine and Hydrogen Peroxide, World Health Organization, 1999.
[4] J.M. Rice, P. Boffetta, 1,3-Butadiene, isoprene and chloroprene reviews by the IARC monographs programme, outstanding issues, and research priorities in epidemiology, Chem. Biol. Interact. 135/136 (2001) 11–26.
[5] G.M. Marsh, A.O. Youk, J.M. Buchanich, M. Cunningham, N.A. Esmen, T.A. Hall, M.L. Phillips, Mortality patterns among industrial workers exposed to chloroprene and other substances. II. Mortality in relation to exposure, Chem. Biol. Interact. 166 (2007) 301–316.
[6] D. Grosjean, Atmospheric chemistry of toxic contaminants. 5. Unsaturated halogenated aliphatics: allyl chloride, chloroprene, hexachlorocyclopentadiene, vinylidene chloride, J. Air Waste Manage. Assoc. 41 (1991) 182–189.
[7] NTP, Toxicology and Carcinogenesis Studies of Chloroprene, (CAS No. 126-99-8), in F344/N Rats and B6C3F1 Mice (Inhalation Studies), National Toxicology Program, NTP TR 467, 1996.
[8] A.E. Nystrom, Health hazards in the chloroprene rubber industry and their prevention, Acta Med. Scand. 132 (Suppl. 219) (1948) 1–125.
[9] C.A. Stewart Jr., Chlorocarbons–hydrocarbons Kirk-Othmer Encyclopedia of Chemical Technology, vol. 6, John Wiley, Indianapolis, IN, 1993, pp. 70–78.
[10] Kirk-Othmer, Chloroprene, in Kirk-Othmer Encyclopedia of Chemical Technology, vol. 6, John Wiley, Indianapolis, IN, 1993, pp. 70-78.
[11] T.J. Haley, Chloroprene (2-chloro-1,3-butadiene)—what is the evidence for its carcinogenicity? Clin. Toxicol. 13 (1978) 153–170.
[12] H. Bartsch, C. Malaveille, A. Barbin, et al., Mutagenic and alkylating metabolites of haloethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: evidence for oxirane formation by P450-linked microsomal mono-oxygenases, Arch. Toxicol. 41 (1979) 249–277.
[13] L. Cottrell, B.T. Golding, T. Munter, W.P. Watson, In vitro metabolism of chloroprene species differences, epoxide stereochemistry and a de-chlorination pathway, Chem. Res. Toxicol. 14 (11) (2001) 1552–1562.
[14] M.W. Himmelstein, S.C. Carpenter, P.M. Hinderliter, T.A. Snow, R. Valentine, The metabolism of beta-chloroprene: preliminary in-vitro studies using liver microsomes, Chem. Biol. Interact. 1 (135/136) (2001) 267–284.
[15] T. Munter, L. Cottrell, B.T. Golding, W.P. Watson, Detoxication pathways involving glutathione and epoxide hydrolase in the in vitro metabolism of chloroprene, Chem. Res. Toxicol. 16 (10) (2003) 1287–1297.
[16] M.W. Himmelstein, S.C. Carpenter, P.M. Hinderliter, Kinetic modeling of beta-chloroprene metabolism. I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans, Toxicol. Sci. 79 (1) (2004) 18–27.
[17] M.W. Himmelstein, S.C. Carpenter, M.V. Evans, P.M. Hinderliter, E.M. Kenyon, Kinetic modeling of beta-chloroprene metabolism. II. The application of physiologically based modeling for cancer dose response analysis, Toxicol. Sci. 79 (1) (2004) 28–37.
[18] R. Dewoskin, PBPK models in risk assessment: a focus on chloroprene, in: Proceedings of the International Symposium on Evaluation of Butadiene and Chloroprene Health, Charleston, SC, USA, September 20–22, 2005 (Platform Presentation).
[19] I.V. Sanotskii, Aspects of the toxicology of chloroprene immediate and long-term effects, Environ. Health. Perspect. 17 (1976) 85–93.
[20] D.A. Savitz, N.L. Sonnenfeld, A.F. Olshan, Review of epidemiologic studies of paternal occupational exposure and spontaneous abortion, Am. J. Ind. Med. 25 (1994) 361–383.
[21] S.D. Schrag, R.L. Dixon, Occupational exposures associated with male reproductive dysfunction, in: R. George, R. Okun (Eds.), Ann. Rev. Pharmacol. Toxicol. 25 (1985) 567–592.
[22] N. Roeleveld, G.A. Zielhuis, F. Gabreëls, Occupational exposure and defects of the central nervous system in offspring, Br. J. Ind. Med. 47 (1990) 580–588 (Review).
[23] P. Amblard, J. Faure, J. Martel, et al., Alopecia in a factory making polychloroprene elastomers, Bulletin de la Societe Francaise de Dermatologie et de Syphiligraphie 81 (1974) 114–115.
[24] W.L. Ritter, A.S. Carter, Hair loss in neoprene manufacture, J. Ind. Hyg. Toxicol. 30 (1948) 192–195.
[25] L. Schwartz, Skin hazards in the manufacture and processing of synthetic rubber, JAMA 127 (1945) 389–391.
[26] H.J. Trochimowicz, E. Löser, V.J. Feron, et al., Chronic inhalation toxicity and carcinogenicity studies on β-chloroprene in rats and hamsters, Inhalation Toxicol. 10 (1998) 443–472.
[27] Q.A. Dong, B.L. Xiao, Y.H. Hu, et al., Short-term test for the induction of lung tumor in mouse by chloroprene, Biomed. Environ. Sci. 2 (1989) 150–153.
[28] J.J. Gooch, W.F. Hawn, Biochemical and hematological evaluation of chloroprene workers, J. Occup. Med. 23 (1981) 268–272.

[29] J.B. Ward Jr., E.B. Whorton, P.R. Gilmer Jr., et al., Liver function among neoprene workers, NTIS/PB82-231044, 1982.
[30] L. Shouqi, D. Quinan, L. Yingfei, Epidemiologic study of cancer mortality among chloroprene workers, Biomed. Environ. Sci. 2 (1989) 141–149.
[31] M.A. Bulbulyan, A.G. Margaryan, S.A. Ilychova, et al., Cancer incidence and mortality in a cohort of chloroprene workers from Armenia, Int. J. Cancer 81 (1999) 31–33.
[32] M.A. Bulbulyan, O.V. Changuina, D.G. Zaridze, et al., Cancer mortality among Moscow shoe workers exposed to chloroprene (Russia), Cancer Causes Control 9 (1998) 381–387.
[33] S. Pell, Mortality of workers exposed to chloroprene, J. Occup. Med. 20 (1) (1978) 21–29.
[34] T.L. Leet, S.G. Selevan, Mortality analysis of workers exposed to chloroprene, Final report for E.I. Du Pont de Nemours and Company, Inc., 1982.
[35] S. Romazini, G. Lavdevant, J.M. Lutz, et al., Mortality study in occupational exposure to chloroprene, Arch. Mal. Prof. 8 (1992) 721–725.
[36] R.L. Melnick, R.C. Sills, C.J. Portier, et al., Multiple organ carcinogenicity of inhaled chloroprene (2-chloro-1,3-butadiene) in F344/N rats and B6C3F1 mice and comparison of dose–response with 1,3-butadiene in mice, Carcinogenesis 20 (5) (1999) 867–878.
[37] R.L. Melnick, M.R. Elwell, J.H. Roycroft, et al., Toxicity of inhaled chloroprene (2-chloro-1,3-butadiene) in F344 rats and B6C3F1 mice, Toxicology 108 (1996) 79–91.
[38] M.D. Shelby, Results of NTP-sponsored mouse cytogenetic studies on 1,3-butadiene, isoprene, and chloroprene, Environ. Health Perspect. 86 (1990) 71–73.
[39] G.A. Westphal, M. Blaszkewicz, M. Leutbecher, et al., Bacterial mutagenicity of 2-chloro-1,3-butadiene (chloroprene) caused by decomposition products, Arch. Toxicol. 68 (1994) 79–84.
[40] E. Vogel, Mutagenicity of chloroprene, 1-chloro-1,3-trans butadiene, 1,4-dichlorobutene-2 and 1,4-dichloro-2,3-epoxybutane in *Drosophila melanogaster*, Mut. Res. 67 (1979) 377–381.
[41] M.W. Himmelstein, N.L. Gladnick, E.M. Donner, R.D. Snyder, R. Valentine, In vitro genotoxicity testing of (1-chloroethenyl)oxirane, a metabolite of beta-chloroprene, Chem. Biol. Interact. 135/136 (2001) 703–713.
[42] U.S.EPA, Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC, EPA/630/P-03/001F, 2005a. Available from: http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283.
[43] M.W. Himmelstein, R.C. Leonard, R. Valentine, Default and Physiologically-Based Modeling Approaches for Cancer Dose Response of β-Chloroprene in Rodents and Humans, International Symposium on Evaluation of Butadiene and Chloroprene Health; September 20–22, 2005; Charleston, South Carolina, USA (Platform Presentation).
[44] H.E. Hurst, Md.Y. Ali, Analyses of hemoglobin N-valine adducts and headspace of (1-chloroethenyl)oxirane in erythrocytes indicate selective detoxification of chloroprene epoxide enantiomers, Chem. Biol. Interact. 166 (2007) 332–340.